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Print

Aug 13, 2002

TITLE: Method, apparatus and kits for sequencing of nucleic acids using multiple dyes

For practising the method shown in FIG. 1B, a suitable kit in accordance with the invention includes at least one container containing a mixture of a plurality of sequencing primers, one for each gene region to be evaluated. The plurality of sequencing primers each comprise a reactive portion which hybridizes with DNA in the sample and a label portion, the label portions of the reagents being different and distinguishable one from the other. Preferably, the detectable labels are fluorescent tags, distinguishable one from the other by their emission or excitation spectra.

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## WEST Search History





DATE: Wednesday, July 05, 2006

Hide?	<u>Set</u> <u>Name</u>	<u>Query</u>	<u>Hit</u> <u>Count</u>
	<i>DB=PGPB,USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ</i>		
<input type="checkbox"/>	L1	mixture near primers	2115
<input type="checkbox"/>	L2	L1 same kit	128
<input type="checkbox"/>	L3	L2 same sequenc\$	55
<input type="checkbox"/>	L4	09/803110	1
<input type="checkbox"/>	L5	L4 and (specific\$ near sense)	0
<input type="checkbox"/>	L6	L4 and specific\$	1
<input type="checkbox"/>	L7	(mixture near (region-specific near primer))	0
<input type="checkbox"/>	L8	(mixture same (region-specific near primer))	19
<input type="checkbox"/>	L9	(mixture same (sequencing primers))	684
<input type="checkbox"/>	L10	L9 same kit	148
<input type="checkbox"/>	L11	L10 same detectable label	3
<input type="checkbox"/>	L12	L11 and (specific\$)	3
<input type="checkbox"/>	L13	L12 and (sense and antisense)	0
<input type="checkbox"/>	L14	L12 and sense	0
<input type="checkbox"/>	L15	L12 and flank\$	0
<input type="checkbox"/>	L16	L11 and (chain terminat\$ or ddNTP or dideoxynucleotide)	3
<input type="checkbox"/>	L17	L11 and (sense or antisense)	0
<input type="checkbox"/>	L18	primer near specific near sense strand	1
<input type="checkbox"/>	L19	primer samd (sense strand and anti-sense strand)	0
<input type="checkbox"/>	L20	primer same (sense strand and anti-sense strand)	1268
<input type="checkbox"/>	L21	primer near (sense strand and anti-sense strand)	165
<input type="checkbox"/>	L22	L21 and specific	165
<input type="checkbox"/>	L23	L22 and sequenc\$	165
<input type="checkbox"/>	L24	L23 and region-specific	3
<input type="checkbox"/>	L25	L21 and region-specific	3
<input type="checkbox"/>	L26	L20 and (region near specific)	250
<input type="checkbox"/>	L27	L21 and (region near specific)	118
<input type="checkbox"/>	L28	L27 and sequenc4	0
<input type="checkbox"/>	L29	L27 and sequenc\$	118
<input type="checkbox"/>	L30	L27 and sequencing reagent	2

<input type="checkbox"/>	L31	candidate gene specific primers	4
<input type="checkbox"/>	L32	gene specific primer	5500
<input type="checkbox"/>	L33	L32 same (sense strand and antisense strand)	10
<input type="checkbox"/>	L34	primer near (sense strand and antisense strand)	124
<input type="checkbox"/>	L35	L34 and DNA region	21
<input type="checkbox"/>	L36	kit same reaction vessel or (kit same (plurality near reaction vessels))	575
<input type="checkbox"/>	L37	L36 same (sequencing reagents)	0
<input type="checkbox"/>	L38	L36 same (sequence-specific reagents)	0
<input type="checkbox"/>	L39	L36 same (sequenc\$)	63
<input type="checkbox"/>	L40	L39 same dideoxynucleotide	0
<input type="checkbox"/>	L41	L36 same dideoxynucleotide	2
<input type="checkbox"/>	L42	L36 same (terminat\$ nucleotide)	11
<input type="checkbox"/>	L43	reaction vessel or (plurality near reaction vessels)	116867
<input type="checkbox"/>	L44	L43 same (dideoxynucleotide or terminat\$ nucleotide)	59
<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ</i>			
<input type="checkbox"/>	L45	(sequenc\$ same (simultaneous\$ near (both strand)))	19
<input type="checkbox"/>	L46	((reaction vessel or vessel or tube or single tube or chamber) same primer)	18623
<input type="checkbox"/>	L47	L46 same (dNTP or deoxynucleotide triphosphate)	3036
<input type="checkbox"/>	L48	L47 same (ddNTP or dideoxynucleotide triphosphate)	139
<input type="checkbox"/>	L49	L48 same (thermostable polymerase or thermal\$ stable polymerase or Taq)	9
<input type="checkbox"/>	L50	L49 same kit	3
<input type="checkbox"/>	L51	L49 and kit	8
<input type="checkbox"/>	L52	L47 and (thermostable polymerase or thermal\$ stable polymerase or Taq)	2296
<input type="checkbox"/>	L53	L47 same(thermostable polymerase or thermal\$ stable polymerase or Taq)	1316
<input type="checkbox"/>	L54	L53 same kit	171
<input type="checkbox"/>	L55	L54 same (ddNTP or dideoxynucleotide triphosphate)	3
<input type="checkbox"/>	L56	L54 and (ddNTP or dideoxynucleotide triphosphate)	14
<input type="checkbox"/>	L57	sequenc\$ near primers or sequencing primers	32592
<input type="checkbox"/>	L58	L57 same (single near vessel or chamber or single tube or single carrier)	175
<input type="checkbox"/>	L59	L58 same (ddNTP or dNTP)	42
<input type="checkbox"/>	L60	L58 same (ddNTP and dNTP)	7
<input type="checkbox"/>	L61	L60 and polymerase	7
<input type="checkbox"/>	L62	L58 same thermostabl\$ polymerase	1
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<input type="checkbox"/>	L65	L64 and L46	15
<input type="checkbox"/>	L66	kit same reaction vessels	584

<input type="checkbox"/>	L67	L66 same primer	135
<input type="checkbox"/>	L68	L66 same (region near primer)	0
<input type="checkbox"/>	L69	L66 same (region-specific near primer)	0
<input type="checkbox"/>	L70	L66 same (primer same (sense and antisense))	0
<input type="checkbox"/>	L71	L66 and (primer same (sense and antisense))	59
<input type="checkbox"/>	L72	L71 and microorganism	15
<input type="checkbox"/>	L73	(primers same (sense and antisense)) same sequenc\$	9145
<input type="checkbox"/>	L74	L73 and microorganism	4530
<input type="checkbox"/>	L75	(primers same (sense and antisense)) same (sequenc\$ near microorganism)	0
<input type="checkbox"/>	L76	(primers same (sense and antisense)) same (sequenc\$ same microorganism)	17
<input type="checkbox"/>	L77	L76 and kit	9
<input type="checkbox"/>	L78	(primers same (sense and antisense) same sequenc\$)	9145
<input type="checkbox"/>	L79	L78 and microorganism	4530
<input type="checkbox"/>	L80	L79 and kit	0
<input type="checkbox"/>	L81	L79 and kit	4260
<input type="checkbox"/>	L82	L81 and (reaction vessels)	131
<input type="checkbox"/>	L83	plurality same reaction vessels	4745
<input type="checkbox"/>	L84	L83 same kit	60
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<input type="checkbox"/>	L86	L81 AND ((PLURALITY OR MULTIPLE) SAME REACTION VESSEL)	15
<input type="checkbox"/>	L87	L78 AND ((PLURALITY OR MULTIPLE) SAME REACTION VESSEL)	71
<input type="checkbox"/>	L88	6017699.pn.	2
<input type="checkbox"/>	L89	6017699.pn. and (sense or antisense)	0
<input type="checkbox"/>	L90	5814442.pn.	2
<input type="checkbox"/>	L91	4683195.pn.	2
<input type="checkbox"/>	L92	L78 AND 39	6174
<input type="checkbox"/>	L93	L79 and L83	7
<input type="checkbox"/>	L94	L81 and (chain terminator or dideoxynucleotide triphosphate)	48
<input type="checkbox"/>	L95	L78 and (chain terminator or dideoxynucleotide triphosphate)	111
<input type="checkbox"/>	L96	6242223.pn.	2

END OF SEARCH HISTORY

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L11: Entry 1 of 3

File: PGPB

Jan 2, 2003

DOCUMENT-IDENTIFIER: US 20030003498 A1

TITLE: Method, apparatus and kits for sequencing of nucleic acids using multiple dyes

Detail Description Paragraph:

[0038] For practising the method shown in FIG. 1B, a suitable kit in accordance with the invention includes at least one container containing a mixture of a plurality of sequencing primers, one for each gene region to be evaluated. The plurality of sequencing primers each comprise a reactive portion which hybridizes with DNA in the sample and a label portion, the label portions of the reagents being different and distinguishable one from the other. Preferably, the detectable labels are fluorescent tags, distinguishable one from the other by their emission or excitation spectra.

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L3: Entry 28 of 55

File: USPT

Feb 11, 2003

DOCUMENT-IDENTIFIER: US RE37984 E

TITLE: Process for analyzing length polymorphisms in DNA regions

## CLAIMS:

12. A kit for performing the method of claim 1, comprising: a) at least one vessel containing an equimolar mixture of primers constituting between 1 and 50 of said primer pairs; b) a vessel containing a polymerizing enzyme suitable for performing a primer-directed polymerase chain reaction; c) a vessel containing the deoxynucleotide triphosphates adenosine, guanine, cytosine and thymidine; d) a vessel containing a buffer solution suitable for performing a polymerase chain reaction, or a concentrate of said buffer solution; e) a vessel containing a template DNA that has a nucleotide sequence including a simple or cryptically simple sequence for assaying positive performance of the method. I add., wherein each simple or cryptically simple DNA sequence comprises at least one trinucleotide motif. I addend..

35. A kit for performing analysis of polymorphism in simple or cryptically simple sequences, comprising: a) at least one vessel containing a mixture of primers constituting 1 to 50 primer parts; wherein each of said primer pairs is composed of a first primer complementary to a nucleotide sequence flanking said simple or cryptically simple DNA sequence on the 5' side of said simple or cryptically simple DNA sequence and a second primer complementary to a nucleotide sequence flanking the simple or cryptically simple DNA sequence on the 3' side of said simple or cryptically simple DNA sequence; wherein said first and second primers each anneal to a single site in said DNA template and wherein the annealing sites are separated by 50 to 500 nucleotides of template DNA; b) a vessel containing a template DNA that has a nucleotide sequence including a simple or cryptically simple sequence for assaying positive performance of the method.. I addend.. I add.

42. A kit for analyzing polymorphism in at least one locus in an DNA sample, comprising: a) at least one vessel containing a mixture of primers constituting between 1 and 50 of said primer pairs; b) a vessel containing a polymerizing enzyme suitable for performing a primer-directed polymerase chain reaction; c) a vessel containing the deoxynucleotide triphosphates adenosine, guanine, cytosine and thymidine; d) a vessel containing a buffer solution for performing a polymerase chain reaction; e) a vessel containing a template DNA comprising i) a simple or cryptically simple nucleotide sequence having a repeat motif length of 3 to 10 nucleotides and ii) nucleotide sequences flanking said simple or cryptically simple nucleotide sequence that are effective for annealing at least one pair of said primers, for assaying positive performance of the method.. I addend.

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[First Hit](#) [Fwd Refs](#) [Previous Doc](#) [Next Doc](#) [Go to Doc#](#)

Generate Collection

Print

L3: Entry 44 of 55

File: USPT

Dec 2, 1997

DOCUMENT-IDENTIFIER: US 5693467 A

TITLE: Mycoplasma polymerase chain reaction testing system using a set of mixed and single sequence primers

Detailed Description Text (30):

The present invention further includes a mycoplasma test kit for detection of the presence of a selectively amplified target segment of a nucleic acid in a nucleic acid mixture. The kit comprises: 1) a first stage primer mixture containing a mixture of at least one single sequence and at least one mixed sequence oligonucleotide primers; 2) a second stage primer mixture, wherein said second stage primer mixture contains a mixture of at least one single sequence and at least one mixed sequence oligonucleotide primers; 3) a first positive control nucleic acid 1; and d) a second positive control nucleic acid 2. For example, the preferred primers and amounts listed above apply to the kit as well. Moreover, the reagents can be provided in a more concentrated form for dilution to the appropriate concentrations and amounts by the end user.

Detailed Description Text (34):

Preferably the kit of the present invention will comprise a plurality of vessels, wherein a first vessel contains a mixture of first stage predetermined primers mixture comprising at least one single sequence and at least one mixed sequence oligonucleotide; a second vessel containing a mixture of second stage predetermined primers, wherein said second stage predetermined primers mixture comprises at least one single sequence oligonucleotide and a least one mixed sequence oligonucleotide; a third vessel containing a first positive control nucleic acid; and a fourth vessel containing a second positive control nucleic acid.

Detailed Description Paragraph Table (1):

TABLE 1 Examples of oligonucleotide primer mixtures suitable for use in the mycoplasma detection kit. Primer Primer Sequence Sequence (All sequences written 5' to 3') Name(s)

	First Stage <u>Primer Mixture</u>	ACACCATGGGAG
(C/T) TGGTAAT, F1, MCGpF11 SEQ ID NO'S: 1-2 CTTC (A/T) TCGACTT (C/T) R1		
CAGACCCAAGGCAT, SEQ ID NO'S 19-22 AAAGTGGGCAATACCCAACGC, M78 SEQ ID NO: 23		
TCACGCTTAGATGCTTTCAGCG M89 SEQ ID NO: 24	Second Stage <u>Primer Mixture</u>	GTG (C/G) GG
(A/C) TGGATCACCTCCT, R16-2, F2 SEQ ID NO'S: 7-10 GCATCCACCA (A/T) A (A/T) AC (C/T)		
CTT, MCGpR21, R2 SEQ ID NO'S: 11-18 CCACTGTGTGCCCTTTGTTTCCT, M34 SEQ ID NO: 25		

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[First Hit](#) [Fwd Refs](#)[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)**End of Result Set**

Generate Collection

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L16: Entry 3 of 3

File: USPT

Aug 13, 2002

DOCUMENT-IDENTIFIER: US 6432634 B1

TITLE: Method, apparatus and kits for sequencing of nucleic acids using multiple dyes

Brief Summary Text (3):

Sequencing of nucleic acids using the chain termination method involves the general steps of combining the target nucleic acid polymer to be sequenced with a sequencing primer which hybridizes with the target nucleic acid polymer; extending the sequencing primer in the presence of normal nucleotide (A, C, G, and T) and a chain-terminating nucleotide, such as a dideoxynucleotide, which prevents further extension of the primer once incorporated; and analyzing the product for the length of the extended fragments obtained. Analysis of fragments may be done by electrophoresis, for example on a polyacrylamide gel.

Brief Summary Text (6):

It is an object of the present invention to provide a further improvement for use with chain termination sequencing reactions which can further increase the throughput of an instrument.

Brief Summary Text (8):

In order to use nucleic acid sequencing as a diagnostic tool, it will be necessary to determine the sequence of the same DNA region from many samples. The present invention makes it possible to increase the throughput of an instrument being used for this purpose. Thus, a first aspect of the invention provides a method for evaluating the sequence of a target nucleic acid polymer in a plurality of samples. In this method, each sample is first divided into four aliquots which are combined with four sequencing reaction mixtures. Each sequencing reaction mixture contains a polymerase enzyme, a primer for hybridizing with the target nucleic acid, nucleotide triphosphate feedstocks and a different dideoxynucleotide triphosphate. This results in the formation of an A-mixture, a G-mixture, a T-mixture and a C-mixture for each sample containing product oligonucleotide fragments of varying lengths. The product oligonucleotide fragments are labeled with fluorescent tags, and these tags will generally be the same for all four sequencing reactions for a sample. However, the fluorescent tags used for each sample are distinguishable one from the other on the basis of their excitation or emission spectra.

Detailed Description Text (3):

As shown in FIG. 1A, two samples, "sample 1" and "sample 2" are each divided into four aliquots and these aliquots are introduced into sequencing reactions A1, C1, G1, and T1, and A2, C2, G2 and T2. Each sequencing reaction contains the reagents necessary for producing product oligonucleotide fragments of varying lengths indicative of the position of one-base within the target nucleic acid sequence. These reagents include a polymerase enzyme, for example T7 polymerase, Sequenase.TM., Thermo Sequenase.TM., or the Klenow fragment of DNA polymerase; A, C, G and T nucleoside feedstocks; one type of chain terminating dideoxynucleoside; and a sequencing primer.

Detailed Description Text (7):



Suitable labels for use in the present invention are fluorescent tags. These can be incorporated into the product oligonucleotide fragments in any way, including the use of fluorescently tagged primers or fluorescently tagged chain terminating reagents.

Detailed Description Text (20):

In a preferred embodiment, a kit in accordance with the invention comprises a plurality of primers for sequencing the selected region, each of the primers having a different and distinguishable fluorescent label. Thus, the reactive portions of the reagents in this case are the oligonucleotide primer to which the labels are attached. The reactive portions may be different from one another, but are preferably the same. Such a kit may also include additional reagents for sequencing, including polymerase enzymes, dideoxynucleotide triphosphate and buffers.

Detailed Description Text (21):

Alternatively, the kit may contain one primer for the selected region and a plurality of containers of chainterminating nucleotide triphosphates, each labeled with a different and distinguishable fluorescent label. In this case, the reactive portion of the reagent is the chain terminating nucleotide triphosphate which can be incorporated in place of a normal nucleotide triphosphate during the sequencing reaction. The kit may include reagents having just one type of chain-terminating nucleotide triphosphate, for example ddA with a plurality of distinct fluorescent labels, or it may include reagents having two or more types of chain-terminating nucleotide triphosphates, each with a plurality of distinct labels. Such a kit may also include additional reagents for sequencing, including polymerase enzymes and buffers, as well as additional chain-terminating nucleotide triphosphates (single-labeled) for those not provided as part of a multi-label reagent set.

Detailed Description Text (22):

For practising the method shown in FIG. 1B, a suitable kit in accordance with the invention includes at least one container containing a mixture of a plurality of sequencing primers, one for each gene region to be evaluated. The plurality of sequencing primers each comprise a reactive portion which hybridizes with DNA in the sample and a label portion, the label portions of the reagents being different and distinguishable one from the other. Preferably, the detectable labels are fluorescent tags, distinguishable one from the other by their emission or excitation spectra.

Other Reference Publication (5):

Sanger et al., "DNA Sequencing with chain-terminating inhibitor", Proc. Natl Acad Sci USA 74: 5463-5467 (1977).

CLAIMS:

1. A method for evaluating the nucleic acid sequence of a plurality of samples comprising the steps of (a) obtaining a first aliquot of each sample; (b) combining the first aliquot of each sample with an aliquot of a first sequencing reaction mixture containing a polymerase enzyme, a primer for hybridizing with the sample, nucleotide triphosphate feedstocks and a first dideoxynucleotide triphosphate to form a first plurality of mixtures of product oligonucleotide fragments, one for each sample, wherein the product oligonucleotide fragments formed from each first aliquot are labeled with different fluorescent tags, said different fluorescent tags being affixed to the primer or to the dideoxynucleotide triphosphate and being distinguishable one from the other on the basis of their excitation or emission spectra; (c) combining the first plurality of mixtures of oligonucleotide products to form a first combined mixture; (d) loading the first combined mixture onto a separation matrix at a first loading site; (e) applying an electric field to cause the oligonucleotide products to migrate within the separation matrix; and (f) detecting the oligonucleotide products having the different fluorescent tags as

they migrate within the separation matrix.

7. A method for evaluating the sequence of a target nucleic acid polymer in a plurality of samples comprising the steps of (a) obtaining four aliquots of each sample; (b) combining the aliquots of each sample with four sequencing reaction mixtures, each sequencing reaction mixture containing a polymerase enzyme, a common primer for hybridizing with the target nucleic acid, nucleotide triphosphate feedstocks and a different dideoxynucleotide triphosphate to form an A-mixture, a G-mixture, a T-mixture and a C-mixture for each sample containing product oligonucleotide fragments of varying lengths, wherein the product oligonucleotide fragments are labeled with fluorescent tags affixed to the primer or to the dideoxynucleotide triphosphate, and the fluorescent tags used for each sample are distinguishable one from the other on the basis of their excitation or emission spectra; (c) combining the A-mixtures, the G-mixtures, the T-mixtures and the C-mixtures for each sample to form a combined A-mixture, a combined G-mixture, a combined t-mixture and a combined C-mixture; (d) loading the combined A-mixture, the combined G-mixture, the combined T-mixture and the combined C-mixture onto a separation matrix at separate loading sites; (e) applying an electric field to cause the product oligonucleotide fragments to migrate within the separation matrix; and (f) detecting the product oligonucleotide fragments having the different fluorescent tags as they migrate within the separation matrix.

8. A method for evaluating the sequence of a plurality of gene regions within a sample comprising the steps of: (a) combining at least a first aliquot of the sample with a sequencing reaction mixture containing a polymerase enzyme, a plurality of sequencing primer species, one sequencing primer species for each gene region, nucleotide triphosphate feedstocks and a first dideoxynucleotide triphosphate to form a first mixture of product oligonucleotide fragments, wherein each of the sequencing primer species specifically hybridizes with a different gene or a different exon of a gene and is labeled with a different detectable label, said different detectable labels being distinguishable one from the other by a detection system; (b) separating the first mixture of product oligonucleotide fragments based upon the size of the fragments; (c) detecting emissions from the separated oligonucleotide fragments for each different detectable label; and (d) evaluating the sequence of each gene region based upon the oligonucleotide fragments detected.

11. The method according to claim 8, wherein four aliquots of sample are combined with four separate sequencing reaction mixtures, each containing a different dideoxynucleotide triphosphate.

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UMBER: 1999-05597 BIOTECHDS  
 TITLE: New hybridization composites;  
           chromosome paint for fluorescence in situ hybridization  
 AUTHOR: Bar-Am I; Garini Y; Cabib D  
 PATENT ASSIGNEE: Applied-Spectral-Imaging  
 LOCATION: Migdal Haemek, Israel.  
 PATENT INFO: US 5871932 16 Feb 1999  
 APPLICATION INFO: US 1998-25131 17 Feb 1998  
 PRIORITY INFO: US 1998-25131 17 Feb 1998  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 OTHER SOURCE: WPI: 1999-166629 [14]  
 AN 1999-05597 BIOTECHDS

AB A new hybridization composite comprises chromosomal paints each being labeled with a different fluorophore or combination of fluorophores, such that an averaged specific activity of highly repetitive sequences in the hybridization composite equals an averaged specific activity of unique sequences in the hybridization composite. Also claimed is a fluorescent in situ hybridization method involving: obtaining a chromosome spread of a species, preparing a hybridization composite containing chromosome paints; denaturing the hybridization composite and subjecting the hybridization composite to conditions for allowing at least part of the unique sequences in the hybridization composite to remain ss; contacting under hybridization conditions the hybridization composite with the spread; washing away excess hybridization composite; and analyzing and presenting images of the now hybridized chromosome spread. A modification of this method is also claimed. The method paints each of the 24 different human chromosomes a different color and detects, identifies and displays a color human karyotype using a simple method. The method can be used for disease diagnosis. (17pp)

L3 ANSWER 5 OF 7 MEDLINE on STN DUPLICATE 3  
 ACCESSION NUMBER: 95047501 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 7959015  
 TITLE: Characterization and chromosomal localization of the human homologue of a rat AMP-activated protein kinase-encoding gene: a major regulator of lipid metabolism in mammals.  
 AUTHOR: Aguan K; Scott J; See C G; Sarkar N H  
 CORPORATE SOURCE: Department of Molecular Medicine, Hammersmith Hospital, Royal Postgraduate Medical School, London, UK.  
 CONTRACT NUMBER: CA-45127 (NCI)  
 SOURCE: Gene, (1994 Nov 18) Vol. 149, No. 2, pp. 345-50.  
           Journal code: 7706761. ISSN: 0378-1119.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-U06454  
 ENTRY MONTH: 199412  
 ENTRY DATE: Entered STN: 10 Jan 1995  
           Last Updated on STN: 10 Jan 1995  
           Entered Medline: 27 Dec 1994

AB AMP-activated protein kinase (AMPK) phosphorylates and inactivates acetyl-CoA carboxylase and beta-hydroxy beta-methylglutaryl-coenzyme A (HMG-CoA) reductase which are the major enzymes involved in fatty acid and lipid biosyntheses. The AMPK gene from rat (rAMPK) has recently been cloned [Carling et al., J. Biol. Chemical 269 (1994) 11442-11448]. In order to study the structure and function of the human AMPK gene (hAMPK), we have cloned the gene, and report in this communication its nucleotide (nt) sequence, tissue distribution and chromosomal location. Our results show that the ORF of hAMPK encodes 552 amino acids (aa) (62.250 kDa) and is highly conserved with rAMPK with identities of 97.3 and 90% at the aa

and nt levels, respectively. The hAMPK gene bears homology to a yeast protein kinase-encoding gene (snf1) that regulates carbohydrate metabolism, and also with three other genes encoding SNF1-like kinases from different plant species, namely *Arabidopsis thaliana*, *Hordeum vulgare* and *Secale cereale*. As determined by fluorescent in situ hybridization of a human metaphase chromosome spread, hAMPK maps to chromosome 1p31. The size of the hAMPK transcript is 8.5 kb and the transcription start point (tsp) is located approx. 46 bp upstream from the ATG codon. While 10-15% of AMPK is alternatively spliced in most tissues of the rat, our RT-PCR analyses of the hAMPK mRNA did not reveal the presence of any alternatively spliced form of the gene in human tissues. An interesting aspect of AMPK is that its expression, unlike in rat liver, could not be detected in human liver, and thus the purported role of the gene in controlling fatty-acid

NE on STN

ACCESSION NUMBER: 79109087 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 153894  
TITLE: Selective excitation of mithramycin or DAPI fluorescence on double-stained cell nuclei and chromosomes.  
AUTHOR: Leemann U; Ruch F  
SOURCE: Histochemistry, (1978 Dec 13) Vol. 58, No. 4, pp. 329-34.  
Journal code: 0411300. ISSN: 0301-5564.  
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 197904  
ENTRY DATE: Entered STN: 15 Mar 1990  
Last Updated on STN: 15 Mar 1990  
Entered Medline: 28 Apr 1979

AB Fluorescence spectra of leukocytes stained by both mithramycin and DAPI showed that the fluorescence of the two dyes can be separated efficiently by using different excitation wavelengths, for instance the 435 nm and the 365 nm mercury lines. In human chromosomes the complementary ("reverse") banding pattern produced by these dyes may thus be observed on double stained chromosome spreads. In plants, for instance in *Anemone blanda*, the two dyes may reveal two different banding patterns. The results of absorption and fluorescence measurements suggest the existence of at least two binding sites, or types, for each dye, with different fluorescent yields and binding strengths.

L13 ANSWER 2 OF 2 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 79091546 EMBASE  
DOCUMENT NUMBER: 1979091546  
TITLE: Selective excitation of mithramycin or DAPI fluorescence on double-stained cell nuclei and chromosomes.  
AUTHOR: Leemann U.; Ruch F.  
CORPORATE SOURCE: Dept. Gen. Bot., Swiss Fed. Inst. Technol., CH-8092 Zurich, Switzerland  
SOURCE: Histochemistry, (1978) Vol. 58, No. 4, pp. 329-334. .  
CODEN: HCMYAL  
COUNTRY: Germany  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 037 Drug Literature Index  
001 Anatomy, Anthropology, Embryology and Histology  
022 Human Genetics  
005 General Pathology and Pathological Anatomy  
LANGUAGE: English

AB Fluorescence spectra of leukocytes stained by both mithramycin and DAPI showed that the fluorescence of the two dyes can be separated efficiently by using different excitation wavelengths, for instance the 435 nm and the 365 nm mercury lines. In human chromosomes the complementary ('reverse') banding pattern produced by these dyes may thus be observed on double stained chromosome spreads. In plants, for instance in *Anemone blanda*, the two dyes may reveal two different banding patterns. The results of absorption and fluorescence measurements suggest the existence of at least two binding sites, or types, for each dye, with different fluorescent yields and binding strengths.

FILE 'MEDLINE, CAPLUS, EMBASE, BIOTECHDS, SCISEARCH' ENTERED AT 12:12:52

ON 05 JUL 2006

L5 1020 S CHROMOSOME SPREAD#  
L6 561 S L5 AND HYBRIDIZ?  
L7 7 S L6 AND (FLUORESC? (5A) (LABEL# OR TAG# OR REPORTER))  
L8 232 DUP REM L6 (329 DUPLICATES REMOVED)  
L9 193 S L8 AND (IN SITU HYBRIDIZATION OR INSITU HYBRIDIZATION)  
L10 0 S L9 AND (REVERSE BANDING)  
L11 99 S REVERSE BANDING  
L12 0 S L8 AND L11  
L13 2 S L5 AND L11  
L14 15 S L11 AND (IN SITU HYBRIDIZATION OR INSITU HYBRIDIZATION)  
L15 5 DUP REM L14 (10 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 12:27:07 ON 05 JUL 2006

L16 0 S L14 AND L5

=>